A

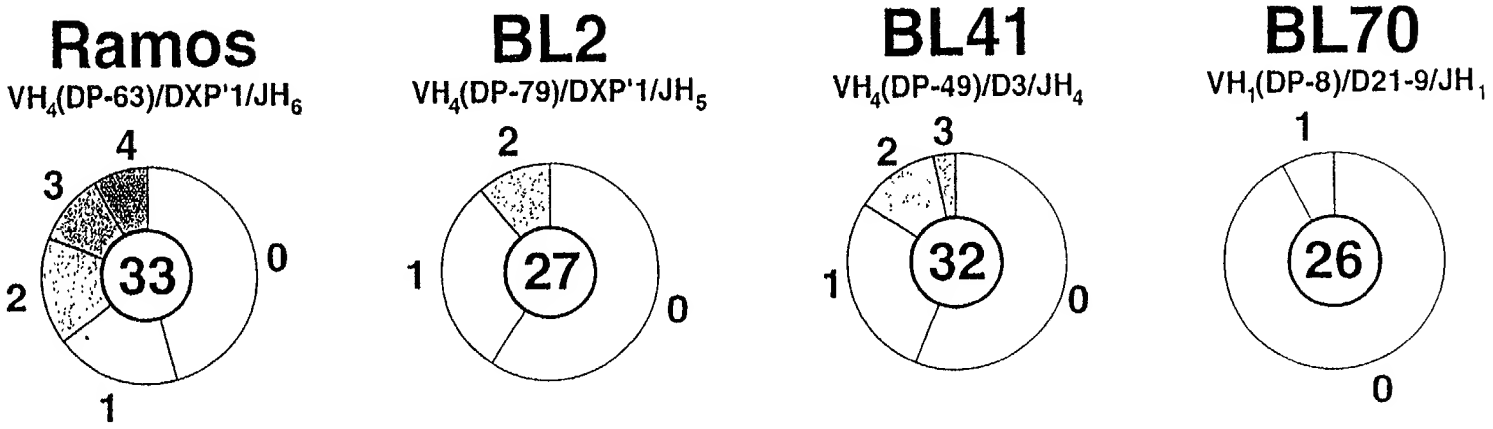


FIG. 1

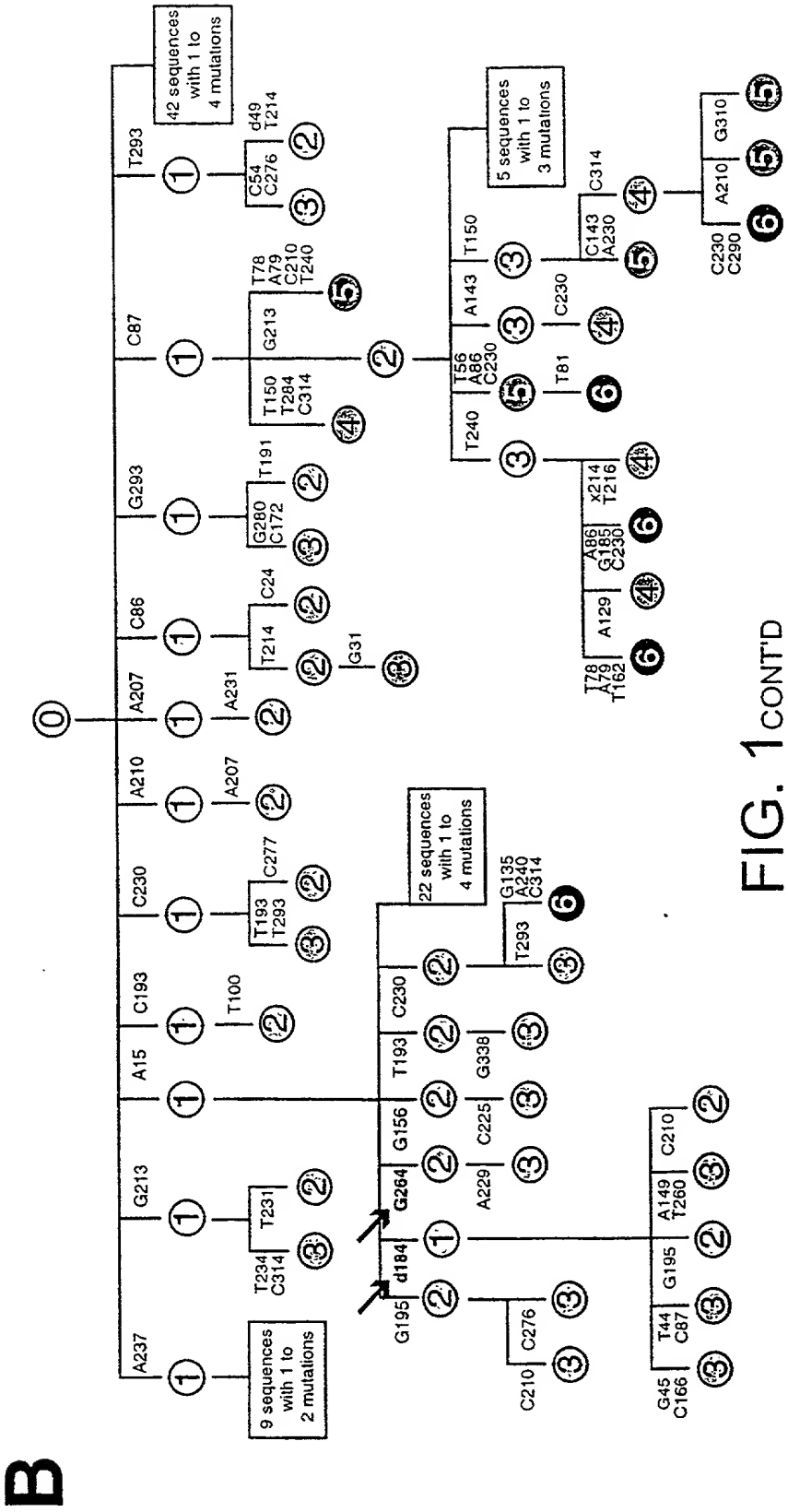
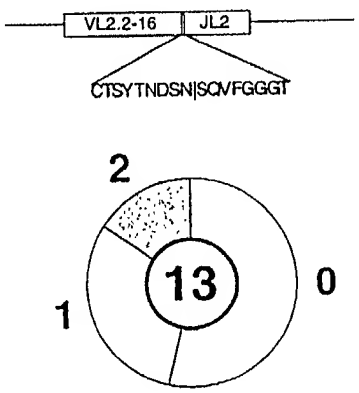


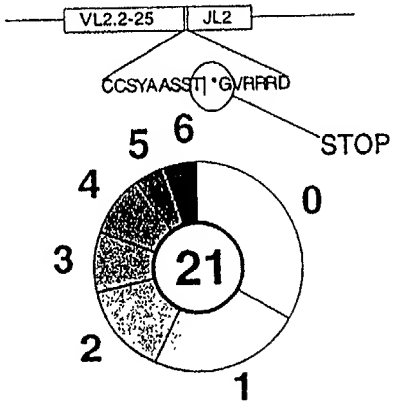
FIG. 1CONT'D

C

In Frame Vλ



Out of Frame Vλ



D

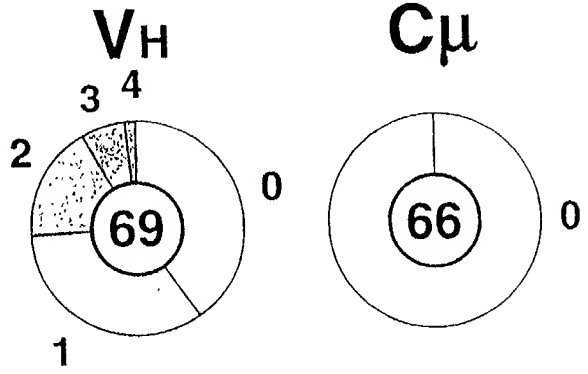
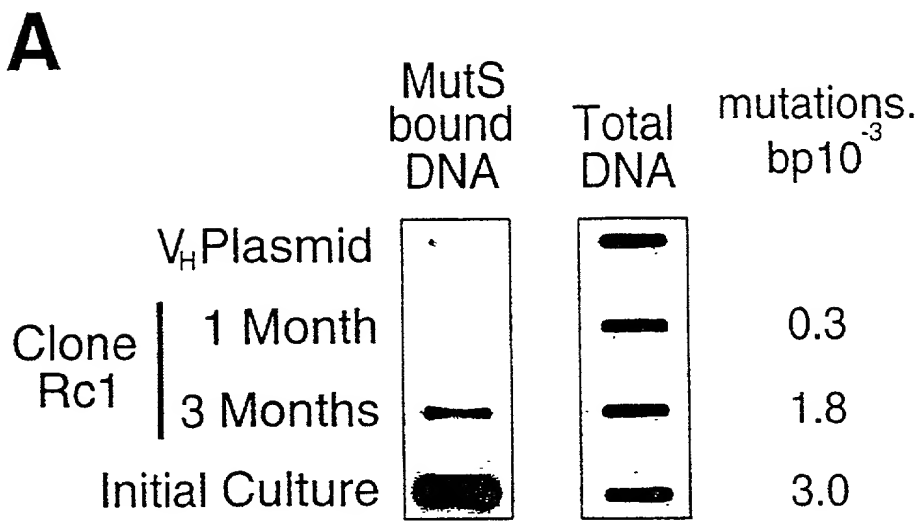
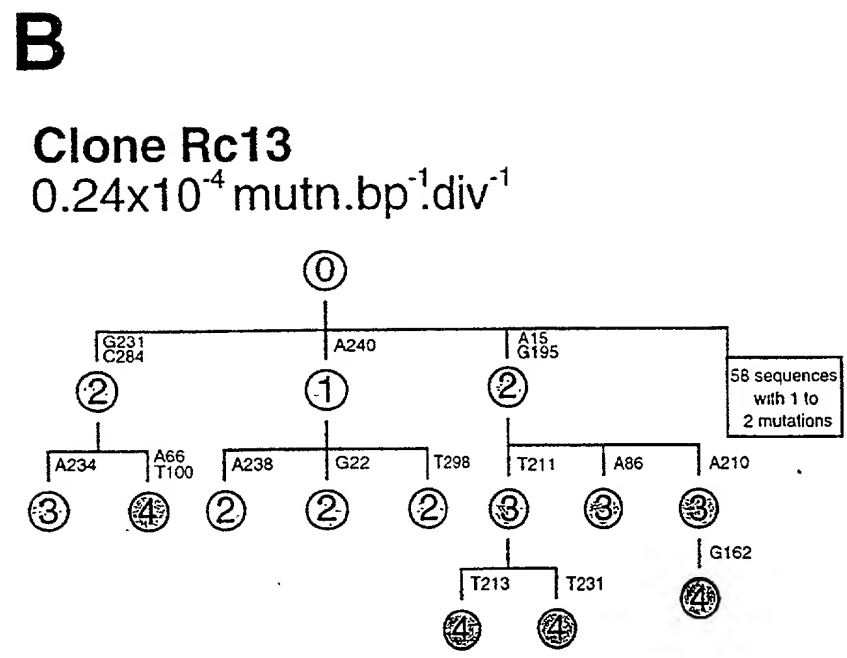
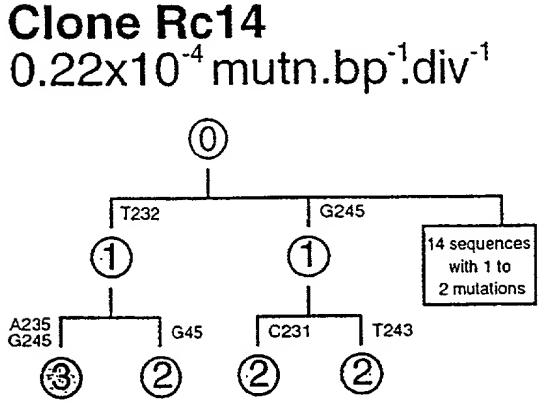


FIG. 1CONT'D


A
MutS bound DNA
Total DNA
mutations. bp10-3
VH Plasmid
Clone Rc1
1 Month
3 Months
Initial Culture
0.3
1.8
3.0


B
Clone Rc13
0.24x10-4 mutn.bp-1.div-1
G231 C284
A240
A15 G195
58 sequences with 1 to 2 mutations
A234
A66 T100
A238
G22
T298
T211
A86
A210
G162
T213
T231


Clone Rc14
0.22x10-4 mutn.bp-1.div-1
T232
G245
14 sequences with 1 to 2 mutations
A235 G245
G45
C231
T243

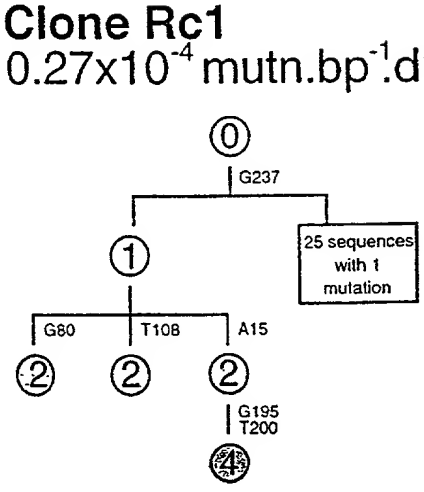

Clone Rc1
0.27x10-4 mutn.bp-1.div-1
G237
25 sequences with 1 mutation
G80
T108
A15
G195 T200

FIG. 2

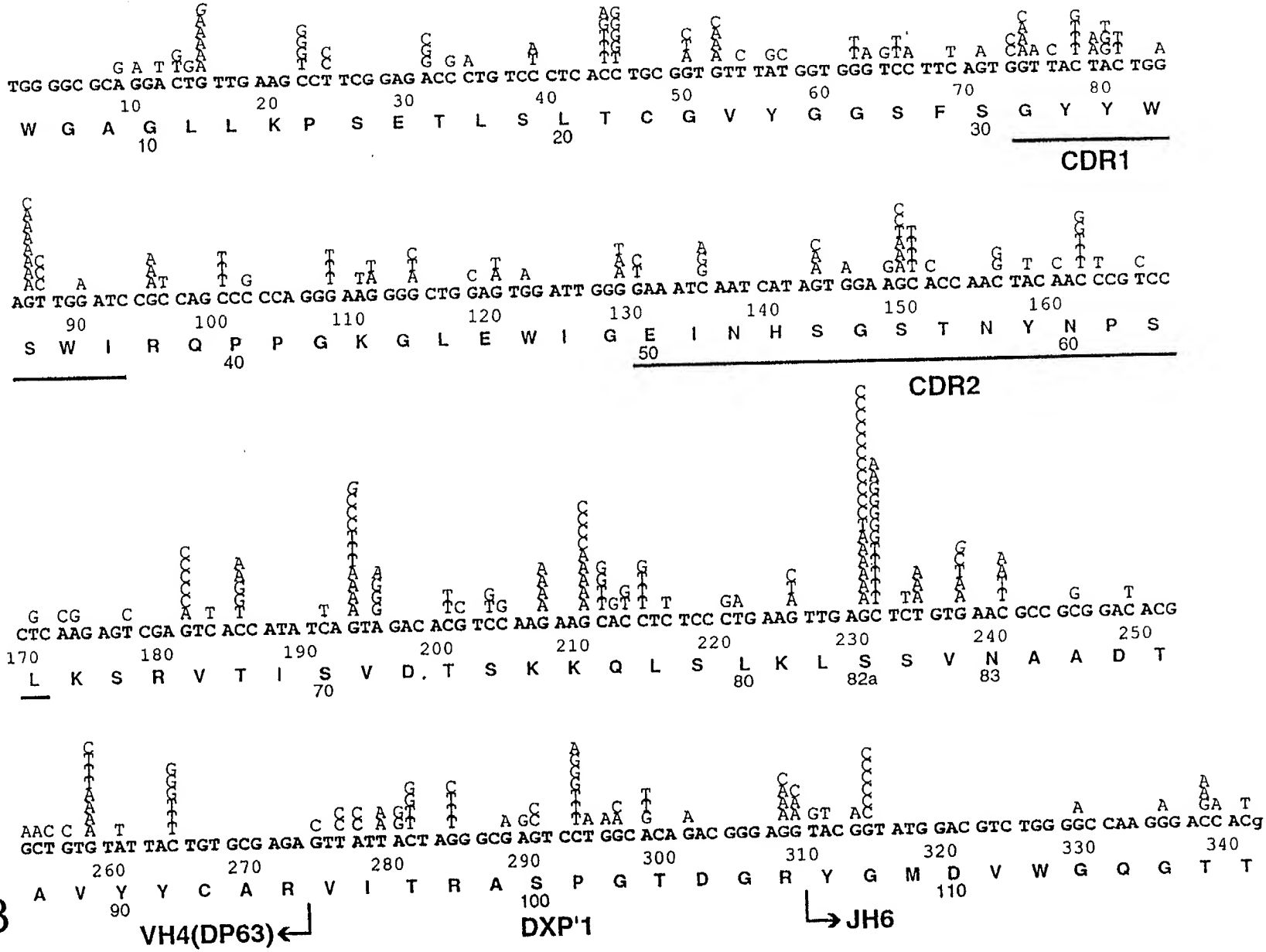

TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT GGG TCC TTC AGT GGT TAC TAC TGG
W G A G L L K P S E T L S L T C G V Y G G S F S G Y Y W
CDR1
AGT TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC
S W I R Q P P G K G L E W I G E I N H S G S T N Y N P S
CDR2
CTC AAG AGT CGA GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAC CTC TCC CTG AAG TTG AGC TCT GTG AAC GCC GCG GAC ACG
L K S R V T I S V D T S K K Q L S L K L S S V N A A D T
GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGC ACA GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACg
A V Y Y C A R V I T R A S P G T D G R Y G M D V W G Q G T T
VH4(DP63)
DXP'1
JH6

FIG. 3

A

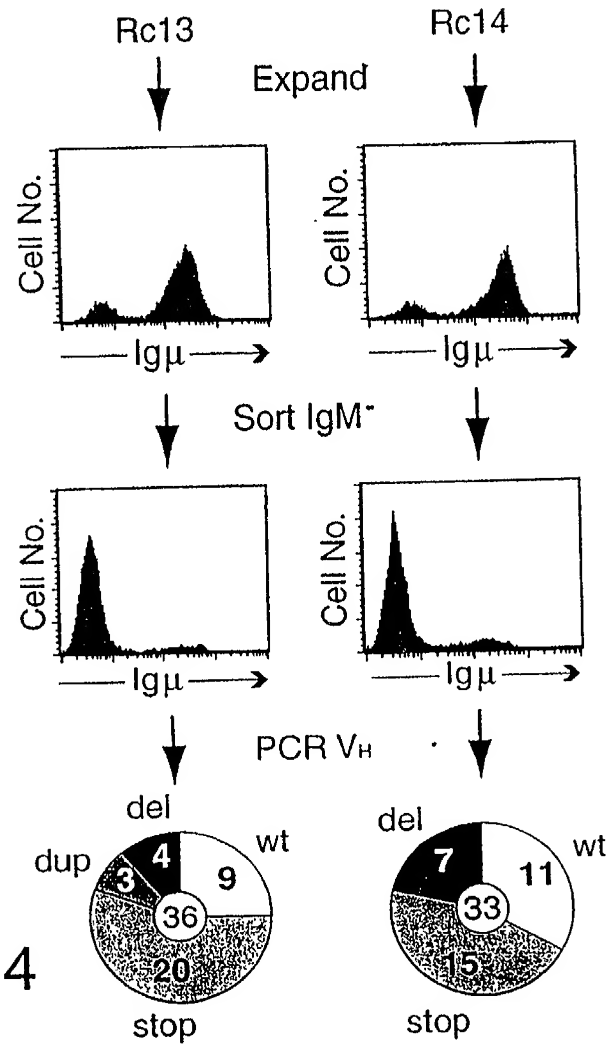


FIG. 4

B

	Rc13	Rc14
TAC 32	TAA (1;1) TAG (5;2)	TAA (1;1) TAG (1;1)
TAC 33	TAG (2;2)	
TGG 34	TGA (1;1)	TGA (1;1)
TGG 36	TGA (6;5)	TGA (1;1)
GAG 46		TAG (1;1)
TGG 47	TAG (1;1)	TGA (1;1)
GAA 50		TAA (2;2)
TCA 70	TGA (2;1)	
TAT 90		TAA (3;2)
TAC 91	TAG (3;1)	TAG (2,2)
TAC106		TAA (1;1)

C

CLONE	% REVN.	STOP	REVN.
Rc13lo.1	1.2%	TAG32	TAC
Rc13lo.2	1.1%	TAG32	TAC TGG AAG
Rc13lo.3	0.08%	TGA36	TGG
Rc13lo.4	0.01%	TAA33	TAC

D

	RGYW
TAG32	GGT**TAG**TAC
TGA36	AGT**TGA**ATC
TAA33	TAC**TAA**TGG

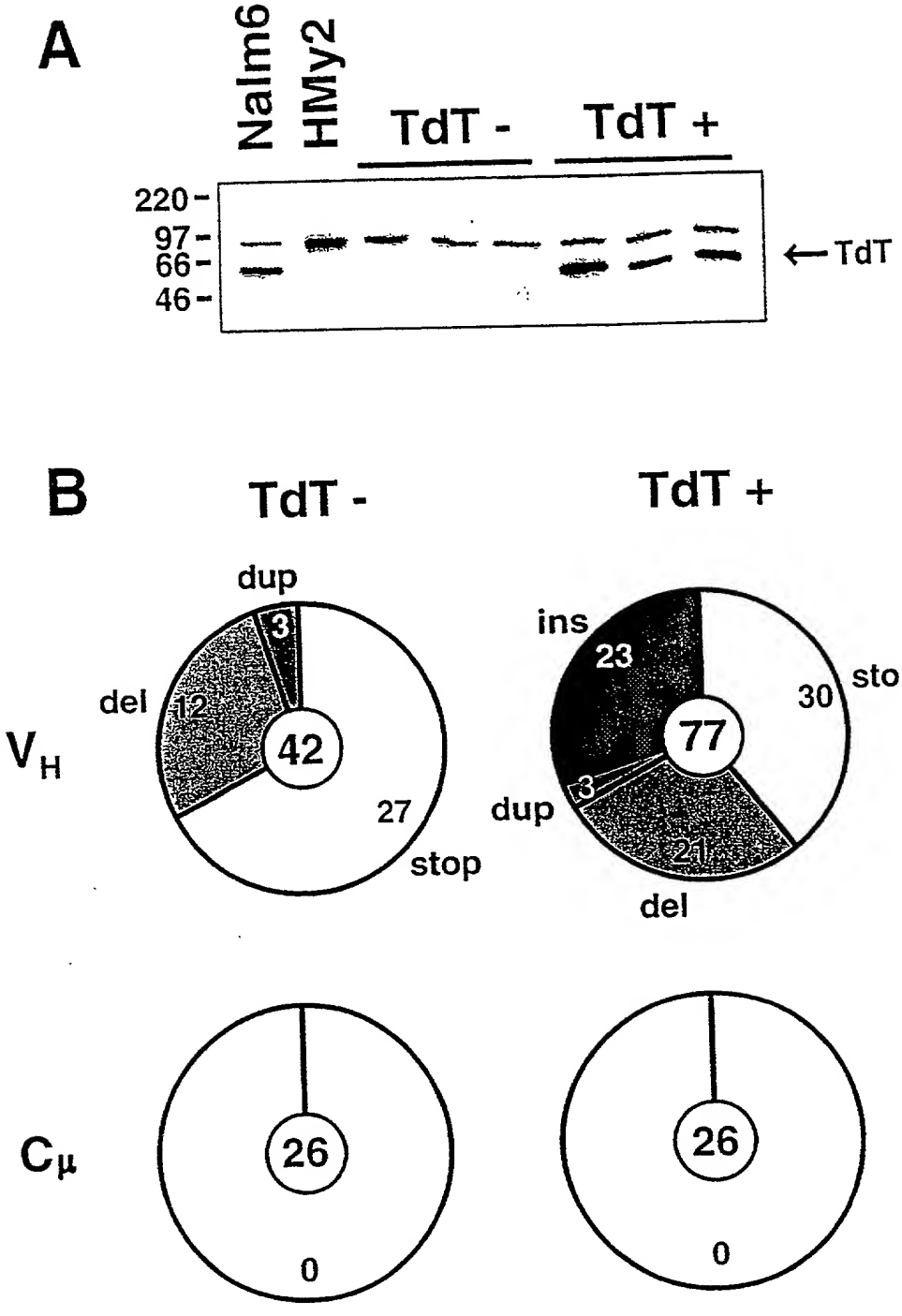

A
Nalm6
HMy2
TdT -
TdT +
220
97
66
46
TdT
B
TdT -
TdT +
dup
3
del
12
42
27
stop
ins
23
30
stop
77
dup
3
21
del
V_H
C_μ
26
0
26
0

FIG. 5

TdT negative

Deletion

A62 GGTCCT^{TCAGTGG}TTACTA

A120 GTGGAT^{T}GGGGAA

A276 TATTAC$^{TGTG.18bp.TACT}$AGGGCG

A306 GAGGTA^{C}GGTATG

B93 CCGCCA^{G}CCCCCA

B98 AGCCCC^{C}AGGGAA

B227 TGAGCT^{CTGTG}AACGCC

C82 TGGAGT$^{TGGA.37bp.GAGT}$GGATTG

C209 AGCACC^{TCTCCCTGAAGTT}GAGCTC

C187 ATATCA^{GTAGACACGTCCAAGA}AGCACC

U26 CGGAGA^{CC}CTGTCC

U199 ACGTCC^{AAG}AAGCAC

U208 AAGCAG^{C}TⓉTCTC

U268 GCGAGA^{GTTATTA}CTAGGG

Duplication

A255 TGTG<u>CGAGAGTTATTA</u><u>CGAGAGTTATTA</u>^{CTAGGG}

A113 GGCTGG<u>AGTGGATTGGG.62bp.T</u> — <u>ATC</u><u>AGTGGATTGGG.62bp.TATC</u>^{AGTAGA}

U43 ACCTGC<u>GGTGTTTAT</u><u>GGTGTTTAT</u>^{GGTGGG}

U318 GG<u>ACGTCTGGGGCCA</u><u>ACGTCTGGGGCCA</u>^{AGGGAC}

TdT positive

Deletion

D27 GGAGAC^{CCTCA}CCTGCG

D31 ACCCTC^{A}CCTGCG

D219 CCTGAA^{G}TTGAGC

D150 CACCAA^{C}TACAAC

D109 AAGGGG^{C}TGGAGT

E38 CCCTCA^{CCTGC}GGTGTT

E81 CTGGAG$^{TTGGA .37bp..TGGAG}$TGGATT

E88 TGGATC^{CGCC}AGCCCC

E93 CCGCCA^{G}CCCCCA

E136 AATCAT^{AGTGGAAGCACCAACTA}CAACCC

F66 CTTCAG^{TGGTTACTACT}GGAGTT

F183 ⓉⒶⓉCAT^{ATCAGTA}ACACGT

F215 TCTCCC$^{TGAA.18bp.CGCC}$GCGGAC

F267 TGCGAG^{AG}TTATTA

Duplication

D55 TATG<u>GTGG.41bp.AGGG</u><u>GTGG.41bp.AGGG</u>^{AAGG}

D123 GATTGGG<u>GAAATCAATCATAGTGGAAGC</u><u>GGAA</u> — <u>ATCAATCATAGTGGAAGC</u>^{ACCAAC}

F85 AGTT<u>GGAT.10bp.CCCA</u><u>GGAT.10bp.CCCA</u>^{GGGA}

Insertion (+/- Del/Dup)

D3 GGⓉCGC$^{AGGACTGT}_{CACCC}$TGAAGC

D56 ATGG<u>TGGG.50bp.CAGGG</u>GG<u>TGGG.50bp.CAGGG</u>^{AAGGGG}

D71 GTGGTT$^{A}_{GGG}$CTACTG

D75 TTACTA$^{C}_{GG}$TGGAGTT

D126 TGGGGA$^{AATCAATCAT}_{GGG}$AGTGGA

D223 AAGTTG$^{AG}_{GACCCGGC}$CTCTGTG

D232 TC<u>TGTGAACGCCGC</u>GCCCCCGGTCC<u>TGTGAACGCCGC</u>^{GGACAC}

D235 GTⒶAAC$_{GGAGG}$^{GCCGCG}

D252 GGCTGT$^{GTATTACTGT}_{TCC}$GCGAGA

D268 GCGAGA$^{GT}_{AGG}$TATⓉATT

D275 TTATTA$^{C}_{GG}$TAGGGC

D332 AAGGGA$^{C}_{AG}$CAC

E3 GGGCGC$^{AGGA.51bp.CTTC}_{GT}$AGTGGT

E51 TGTTTA$^{TGGT.15bp.TACT}_{AGACC}$ACTGGAG

E80 ACTGGA$^{G}_{CCC}$TTGGAT

E263 ACTGTG$^{CGAGAGTTATTACT}_{GGTG}$AGGGCG

F89 GGATCC$^{GCCAGCCCCCAGGG}_{AGG}$AAGGGG

F168 CCTCAA$^{AGAGTCGAGT}_{GGG}$CACCAT

F195 AGACAC$^{GTCCAAGAAG}_{AGGGC}$CACCTC

F199 ACGTCC$^{AAGAAG}_{CT}$ACCCTGA

F242 CCGCGG$^{ACACGGCTGTGTATTACTGT}_{GGA}$GCGAGA

F260 ATTACT$^{GTG}_{CGTGA}$CGAGAG

F264 CTGTGC$^{GAGAG.46bp.CGTC}_{ACA}$TGGGGC

Events with flanking single nucleotide substitutions

Deletion

B123 GATTGG^{G}ⒶAAATC

C109 AAGGGⓉ^{C}TGGAGT

Duplication

A16 TT<u>GAAGCCTTCGGAGⓉ</u><u>GAAGCCTTCGGAGA</u>^{CCCTGT}

U180 AGTC<u>ACCATATCAⓃ</u><u>ACCATATCAG</u>^{TAGACA}

Deletion

D45 CTGCGGⒼ^{GTTTATGGTGGGT}CCTTCA

D164 CGTCCCⒸ^{CAAG}AGTCGA

D216 CTCCCTⓉ$^{AAG.22bp.CGGA}$CACGGC

E11 GACTGT^{T}ⒶAAGCC

E54 TTATGGⒼ$^{GGG.25bp.GTTG}$GATCCG

F188 TATCAGⒼ^{AGACACGTCCAGAA}GCACCT

F220 CTGAAGⒸ^{TGAGCTCTGTG}AACGCC

FIG. 6

1/1
TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG
W G A G L L K P S E

31/11
ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
T L S L T C G V Y G

61/21
GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG
G S F S G Y Y W S W
AGT
S

91/31
ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
I R Q P P G K G L E

121/41
TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC
W I G E I N H S G S

151/51
ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
T N Y N P S L K S R

181/61
GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG
V T I S V D T S K K
ATC
M

211/71
CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
H L S L K L S S V N
CAC
H

241/81
GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG
A A D T A V Y Y C A
TCG
S

271/91
AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
R V I T R A S P G T
ACG
T
CAT GGC
H G

301/101
GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC
D G R Y G M D V W G
GTT
V

331/111
CAA GGG ACC ACG
Q G T T

FIG. 7

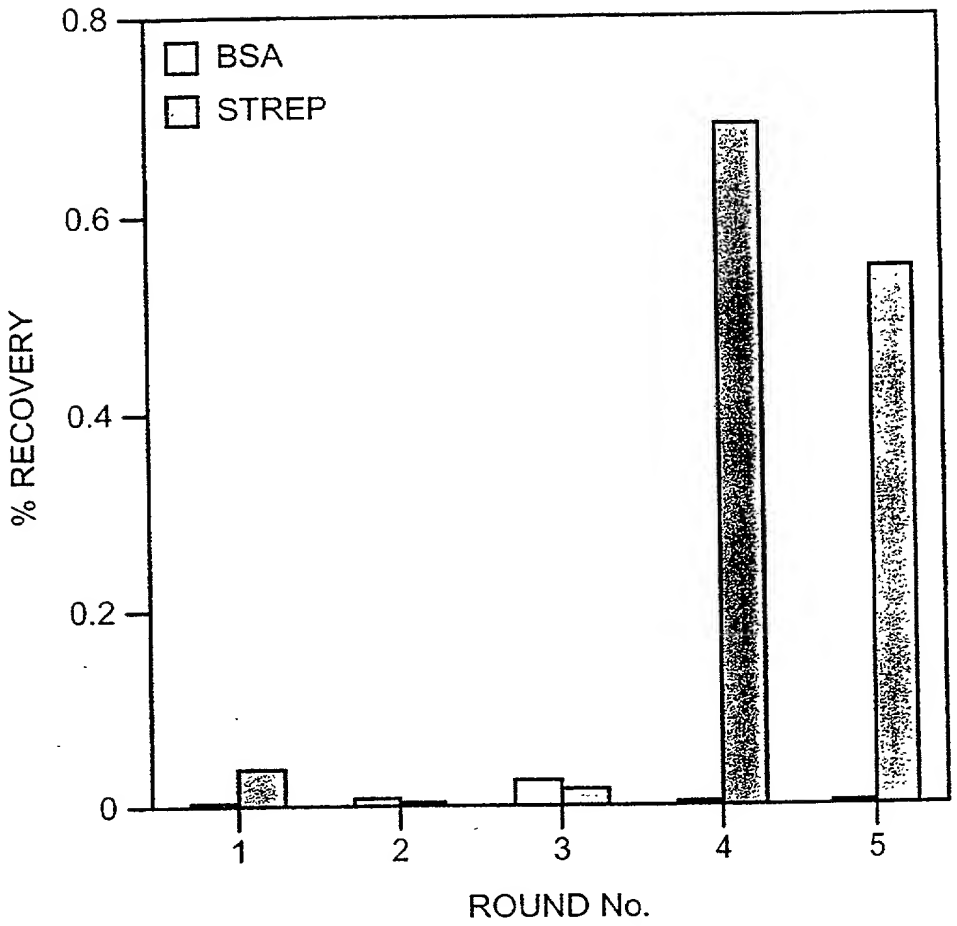


FIG. 8

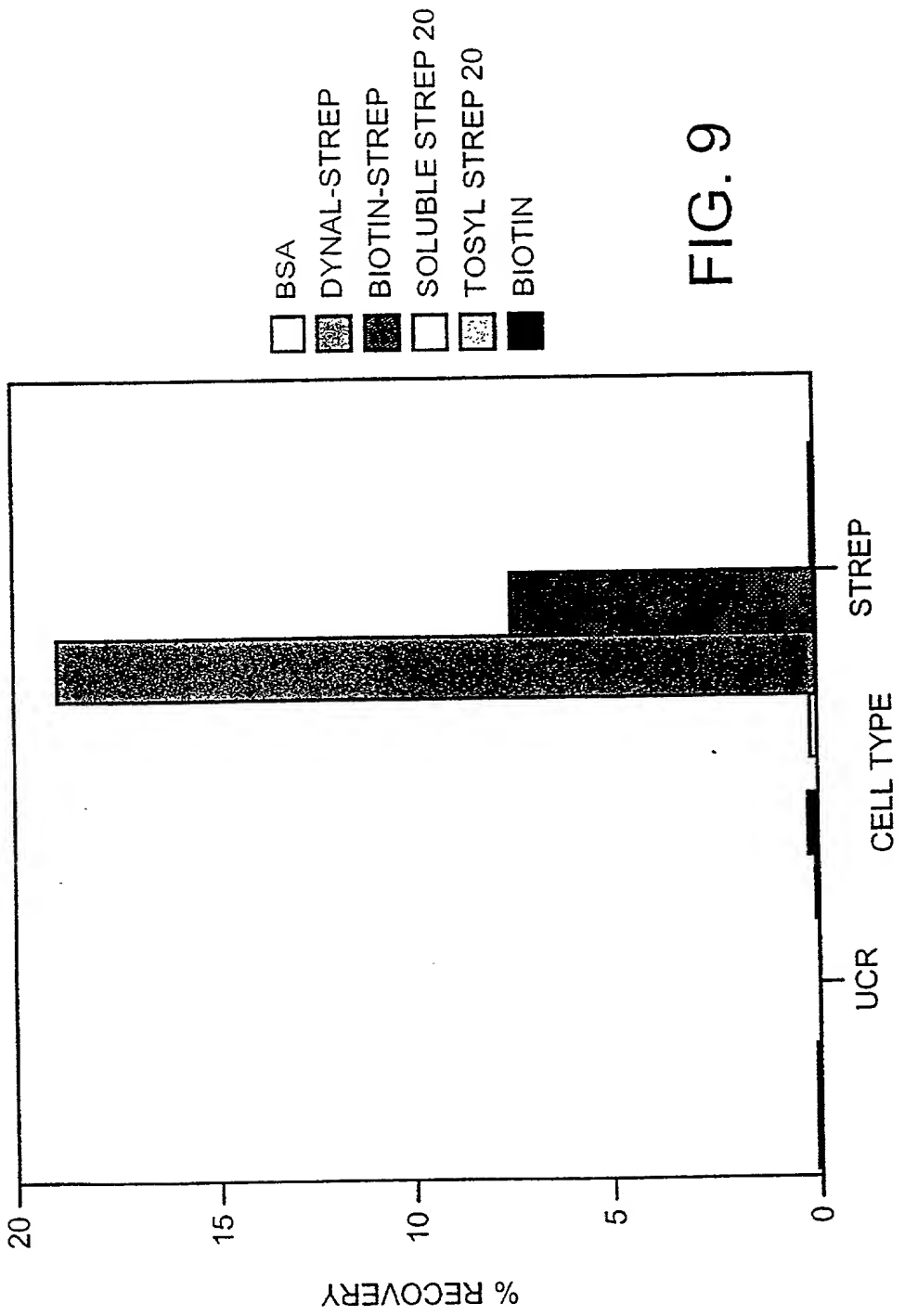

BSA
DYNAL-STREP
BIOTIN-STREP
SOLUBLE STREP 20
TOSYL STREP 20
BIOTIN
% RECOVERY
0
5
10
15
20
UCR
STREP
CELL TYPE

FIG. 9

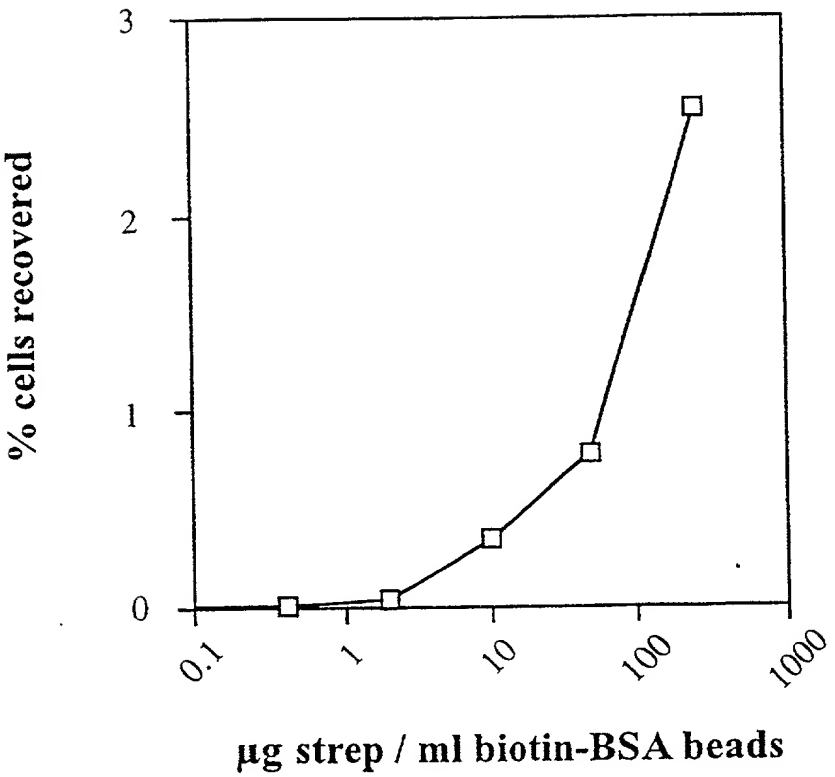


FIG. 10

FIG. 11

VH

1/1
TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG
W G A G L L K P S E

31/11
ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
T L S L T C G V Y G

61/21
GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG
G S F S G Y Y W S W
GG**A**
G
AG**T**
S
A*T***T**
I

91/31
ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
I R Q P P G K G L E

121/41
TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC
W I G E I N H S G S

151/51
ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
T N Y N P S L K S R

181/61
GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG
V T I S V D T S K K

211/71
CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
H L S L K L S S V N
CA**C**
H
AA**C**
N

241/81
GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG
A A D T A V Y Y C A

271/91
AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
R V I T R A S P G T

301/101
GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC
D G R Y G M D V W G
AG**C**
S

331/111
CAA GGG ACC ACG
Q G T T

VL

```
1/1                                               31/11
CCT GCC TCC GTG TCT GGG TCT CCT GGA CAG TCG ATC ACC ATC TCC TGC ACT GGA ACC AGC
P   A   S   V   S   G   S   P   G   Q   S   I   T   I   S   C   T   G   T   S
                TAT
                Y
61/21                                             91/31
AGT GAC GTT GGT GGT TAT AAC TAT GTC TCC TGG TAC CAA CAA AAC CCA GGC AAA GCC CCC
S   D   V   G   G   Y   N   Y   V   S   W   Y   Q   Q   N   P   G   K   A   P
        TTT TGT
        F   C
121/41                                            151/51
AAA CTC ATG ATT TAT GAT GTC AGT AAT CGG CCC TCA GGG ATT TCT AAT CGC TTC TCT GGC
K   L   M   I   Y   D   V   S   N   R   P   S   G   I   S   N   R   F   G   S
                            AAT                                 CGA TTA
                            N                                   R   L
181/61                                            211/71
TCC AAG TCT GGC AAC ACG GCC TCC CTG ACC ATC TCT GGG CTC CAG GCT GAC GAC GAG GCT
S   K   S   G   N   T   A   S   L   T   I   S   G   L   Q   A   D   D   E   A
                                    ATC
                                    I
241/81                                            271/91
GAT TAT TAC TGC ACC TCA TAT ACA AAC GAC AGC AAT TCT CAG GTA TTC GGC GGA GGG ACC
D   Y   Y   C   T   S   Y   T   N   D   S   N   S   Q   V   F   G   G   G   T
                            ACT
                            T
```

FIG. 11 CONT'D

FIG. 12

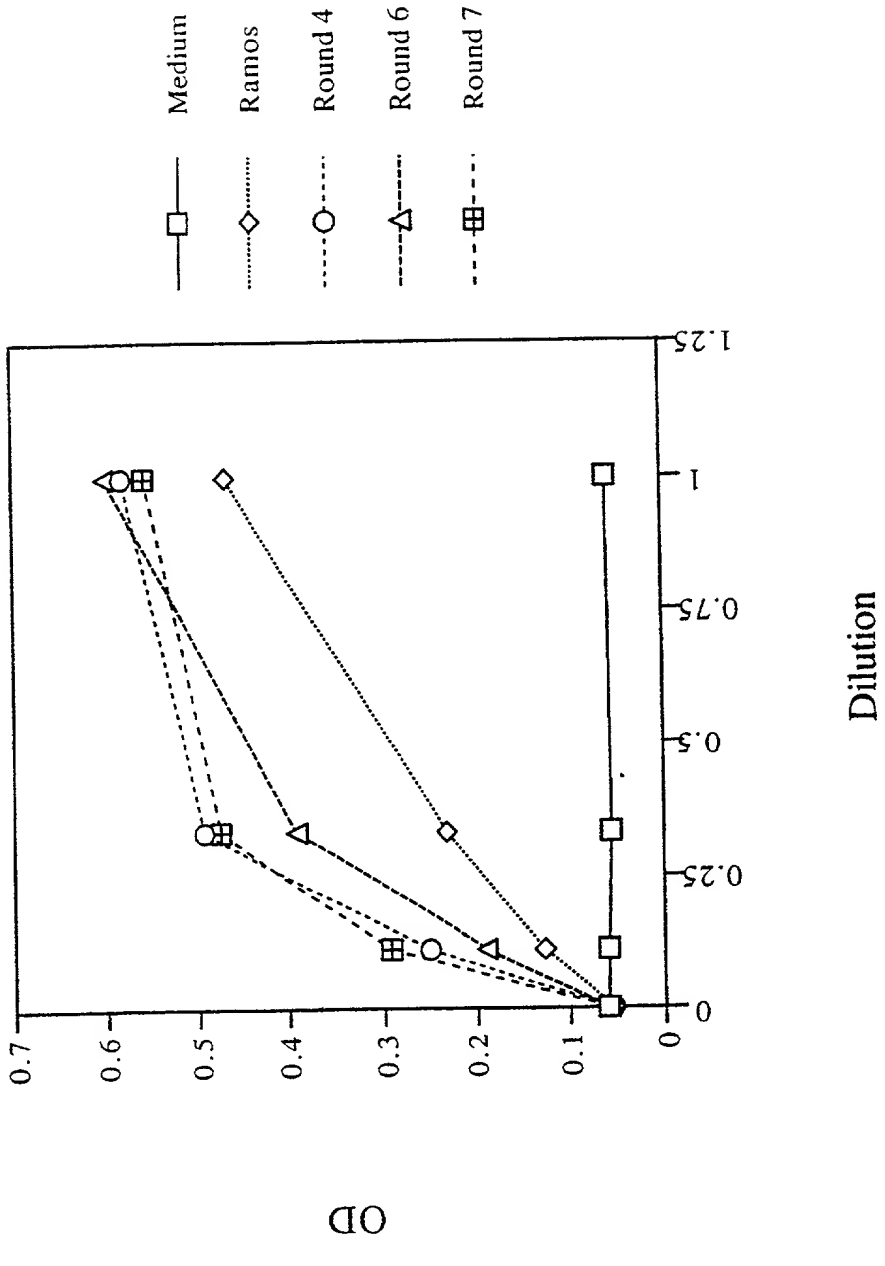

IgM in supernatant
OD
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.25
0.5
0.75
1
1.25
Dilution
Medium
Ramos
Round 4
Round 6
Round 7

FIG. 13

Streptavidin binding of Supernatants: ELISA

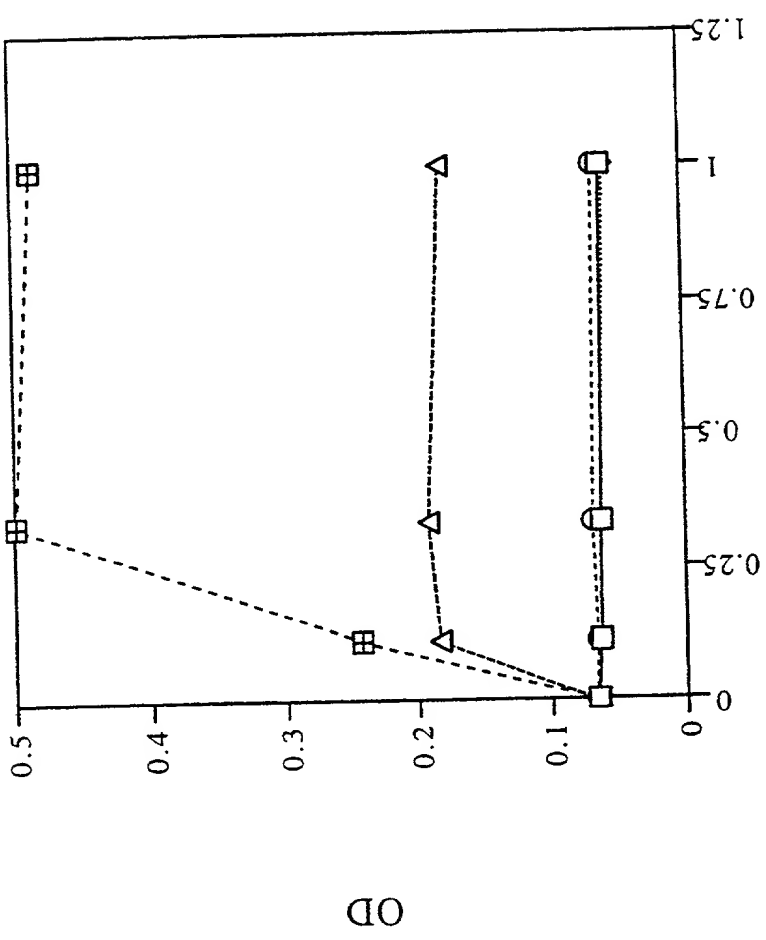
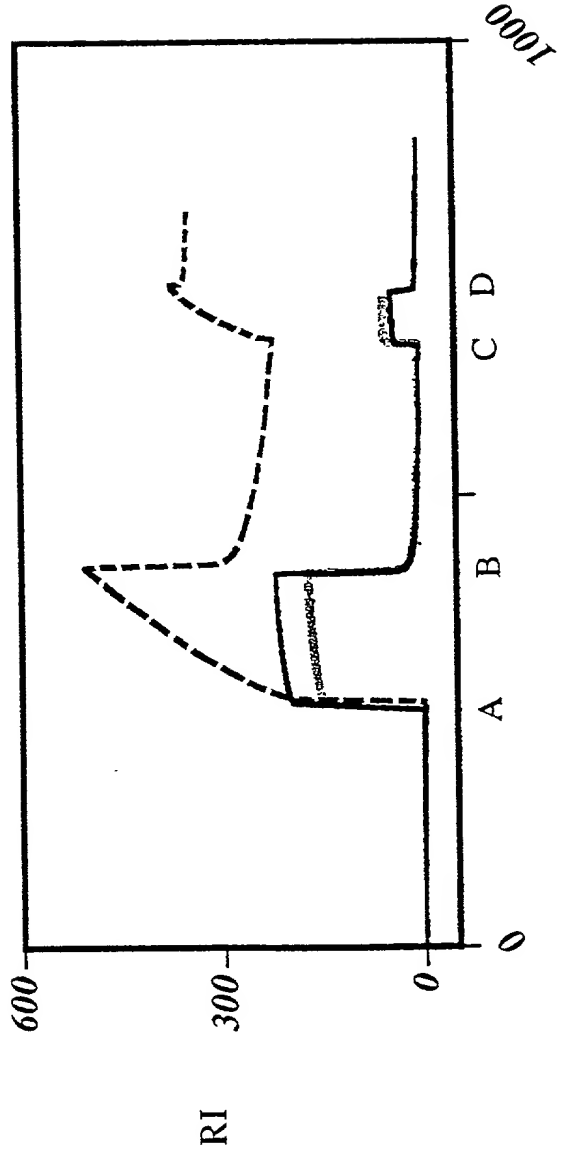


FIG. 14

Streptavidin binding of Supernatants: Biacore



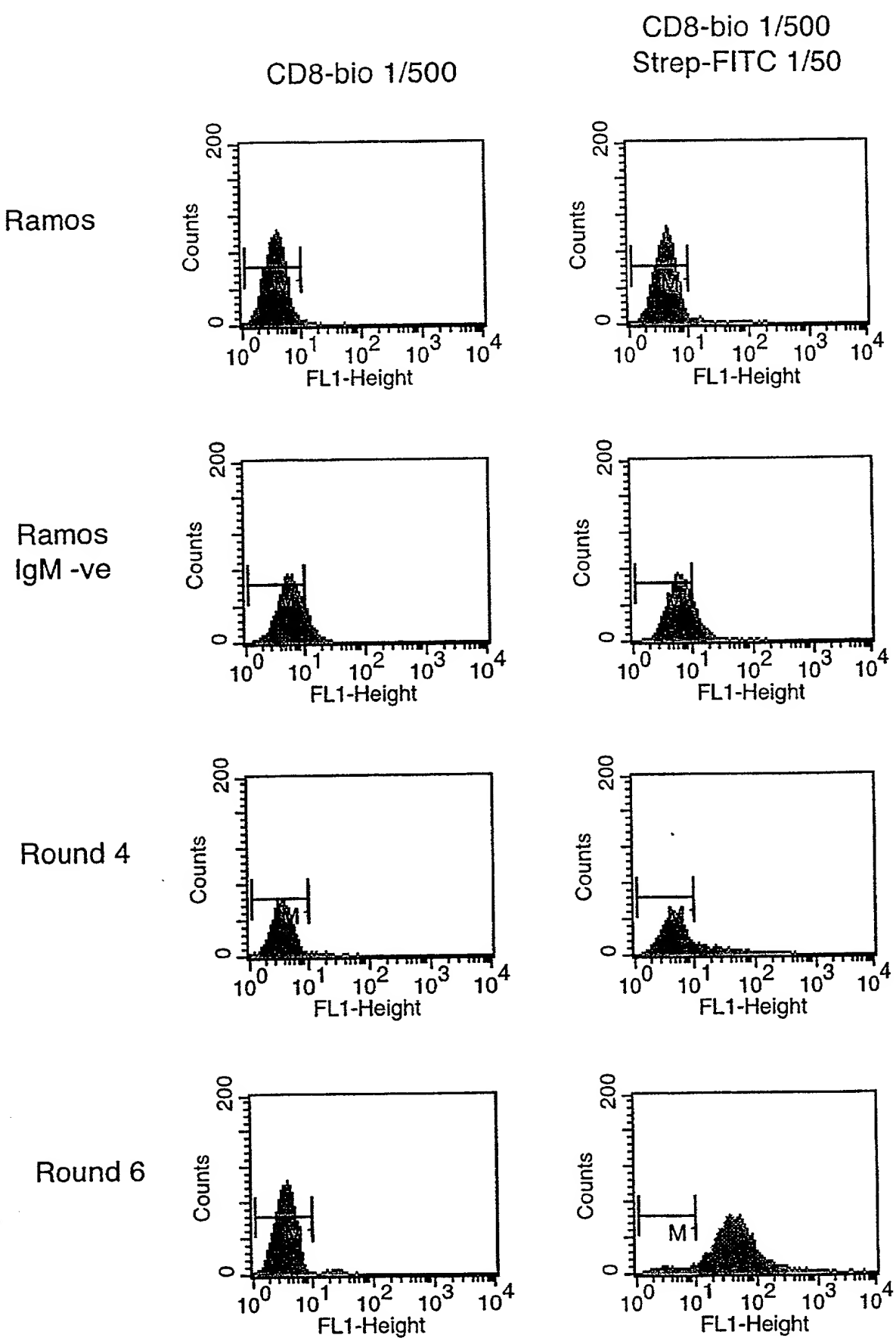


FIG. 15

FIG. 16

VH

1/1 31/11

TGG GGC GCA GGA CTG TTG AAG CCT TCG GAG ACC CTG TCC CTC ACC TGC GGT GTT TAT GGT
W G A G L L K P S E T L S L T C G V Y G

61/21 CDR1 91/31

GGG TCC TTC AGT GGT TAC TAC TGG AGC TGG ATC CGC CAG CCC CCA GGG AAG GGG CTG GAG
G S F S G Y Y W S W I R Q P P G K G L E

121/41 CDR2 151/51

TGG ATT GGG GAA ATC AAT CAT AGT GGA AGC ACC AAC TAC AAC CCG TCC CTC AAG AGT CGA
W I G E I N H S G S T N Y N P S L K S R

181/61 211/71

GTC ACC ATA TCA GTA GAC ACG TCC AAG AAG CAG CTC TCC CTG AAG TTG AGC TCT GTG AAC
V T I S V D T S K K H L S L K L S S V N

241/81 271/91 DJ

GCC GCG GAC ACG GCT GTG TAT TAC TGT GCG AGA GTT ATT ACT AGG GCG AGT CCT GGA ACA
A A D T A V Y Y C A R V I T R A S P G T

301/101 331/111

GAC GGG AGG TAC GGT ATG GAC GTC TGG GGC CAA GGG ACC ACG
D G R Y G M D V W G Q G T T
AGC
S

VL

```
1/1                                     31/11                   CDR1
CCT GCC TCC GTG TCT GGG TCT CCT GGA CAG TCG ATC ACC ATC TCC TGC ACT GGA ACC AGC
P   A   S   V   S   G   S   P   G   Q   S   I   T   I   S   C   T   G   T   S

61/21                                   91/31
AGT GAC GTT GGT GGT TAT AAC TAT GTC TCC TGG TAC CAA CAA AAC CCA GGC AAA GCC CCC
S   D   V   G   G   Y   N   Y   V   S   W   Y   Q   Q   N   P   G   K   A   P
        TTT TGT
        F   C

121/41              CDR2               151/51
AAA CTC ATG ATT TAT GAT GTC AGT AAT CGG CCC TCA GGG ATT TCT AAT CGC TTC TCT GGC
K   L   M   I   Y   D   V   S   N   R   P   S   G   I   S   N   R   F   G   S
                    GCT
                    A

181/61                                  211/71
TCC AAG TCT GGC AAC ACG GCC TCC CTG ACC ATC TCT GGG CTC CAG GCT GAC GAC GAG GCT
S   K   S   G   N   T   A   S   L   T   I   S   G   L   Q   A   D   D   E   A

241/81          CDR3                    271/91
GAT TAT TAC TGC ACC TCA TAT ACA AAC GAC AGC AAT TCT CAG GTA TTC GGC GGA GGG ACC
D   Y   Y   C   T   S   Y   T   N   D   S   N   S   Q   V   F   G   G   G   T
```

FIG. 16CONT'D

In Vitro Maturation

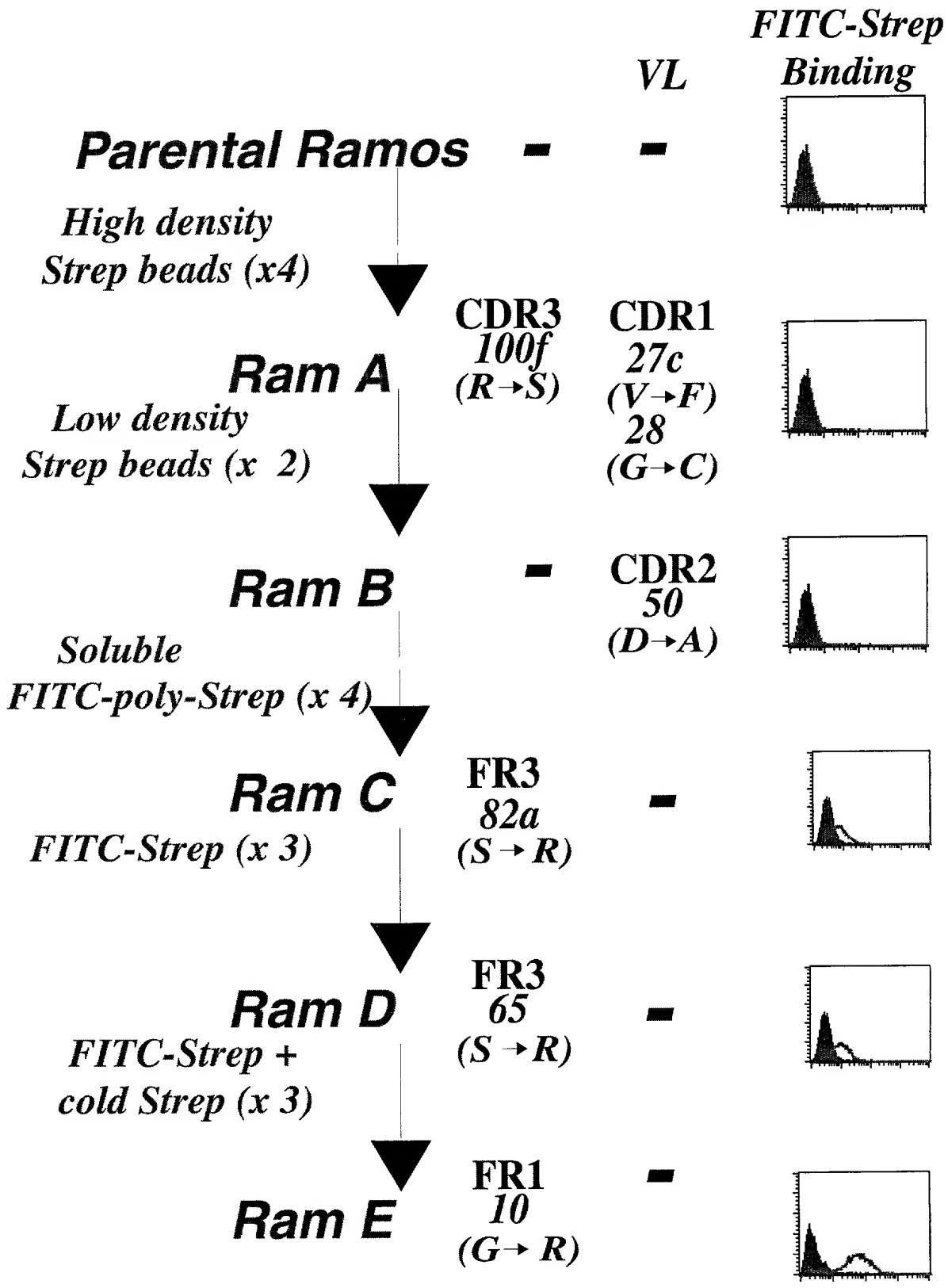


FIGURE 17

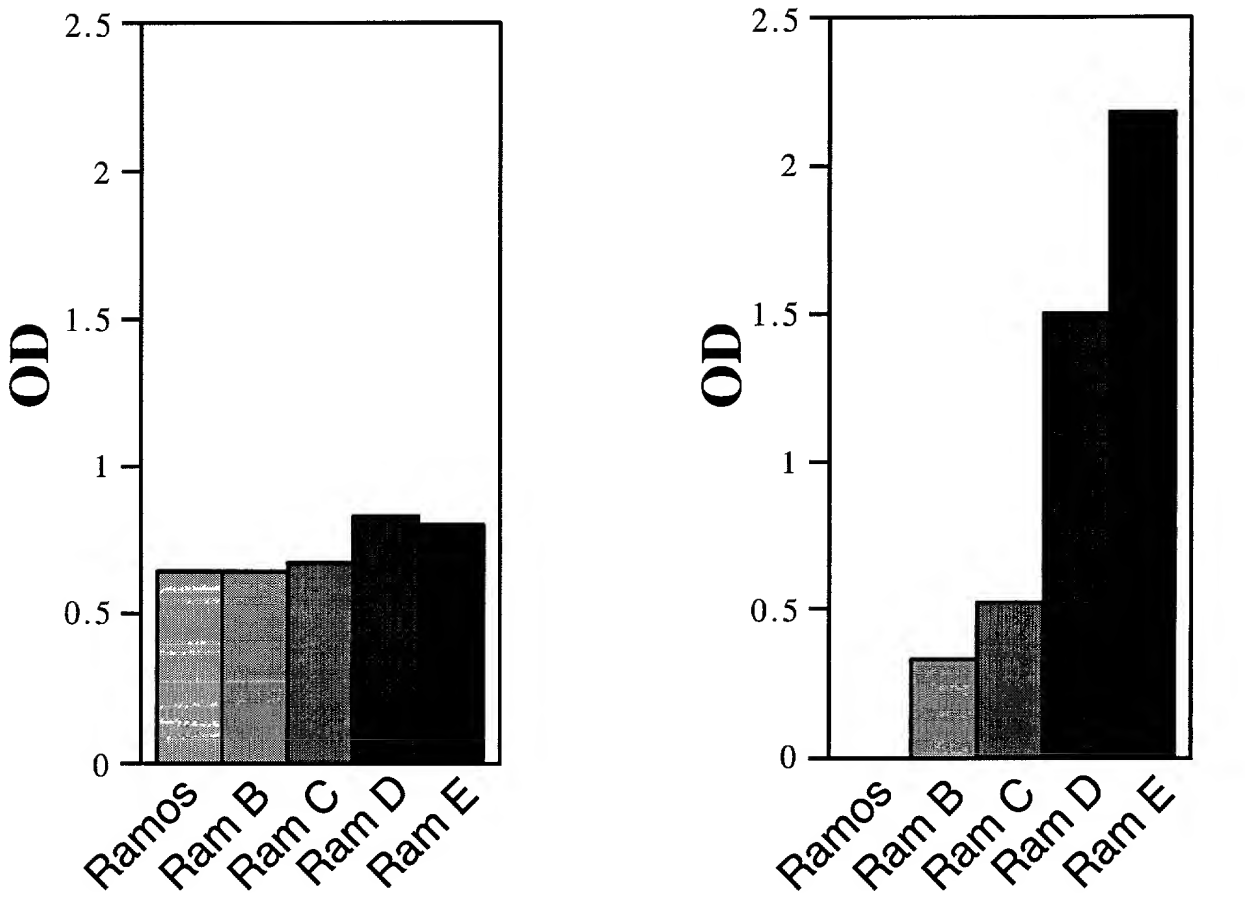
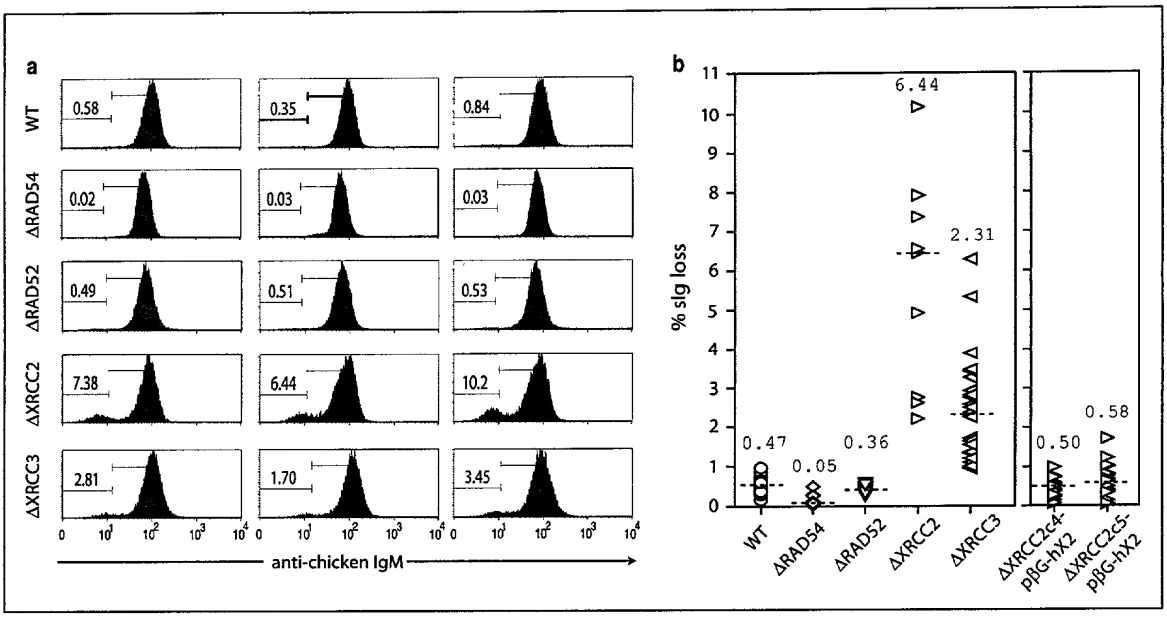
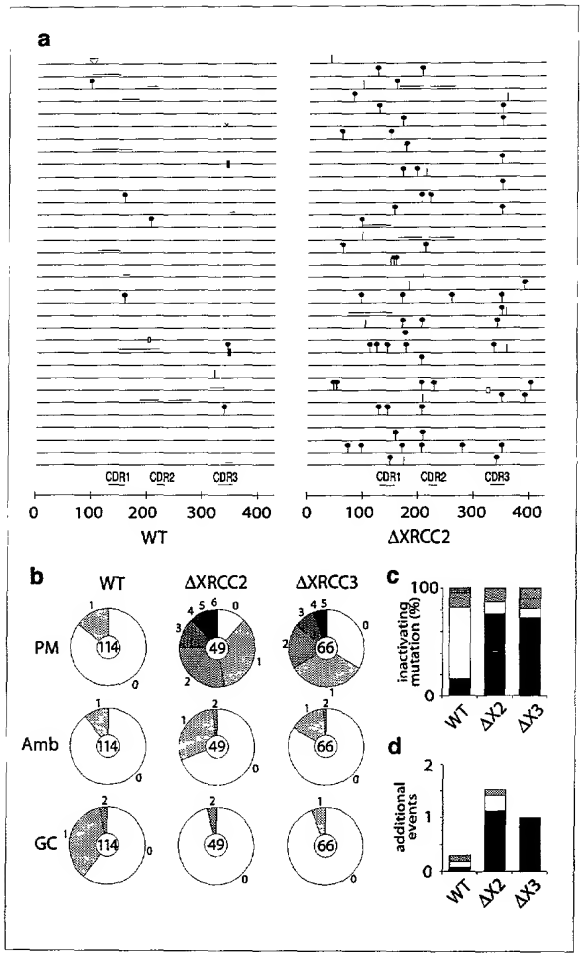


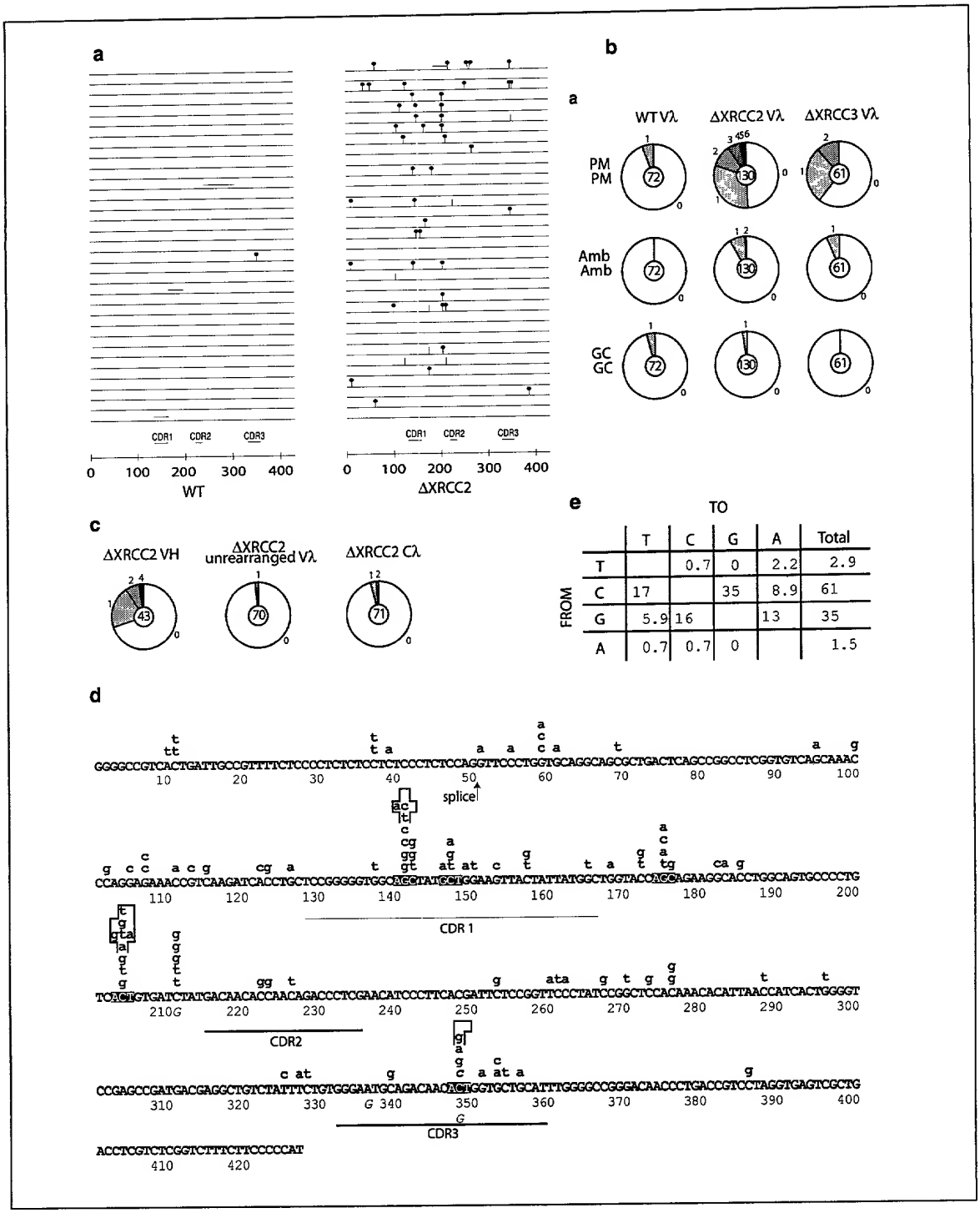
FIGURE 18



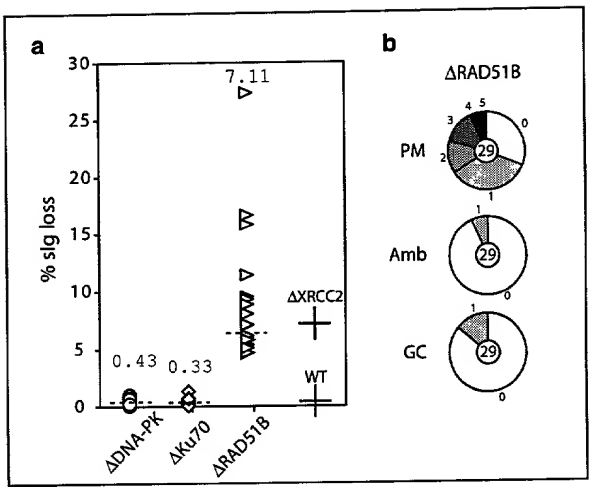
FIGURES 19A-B



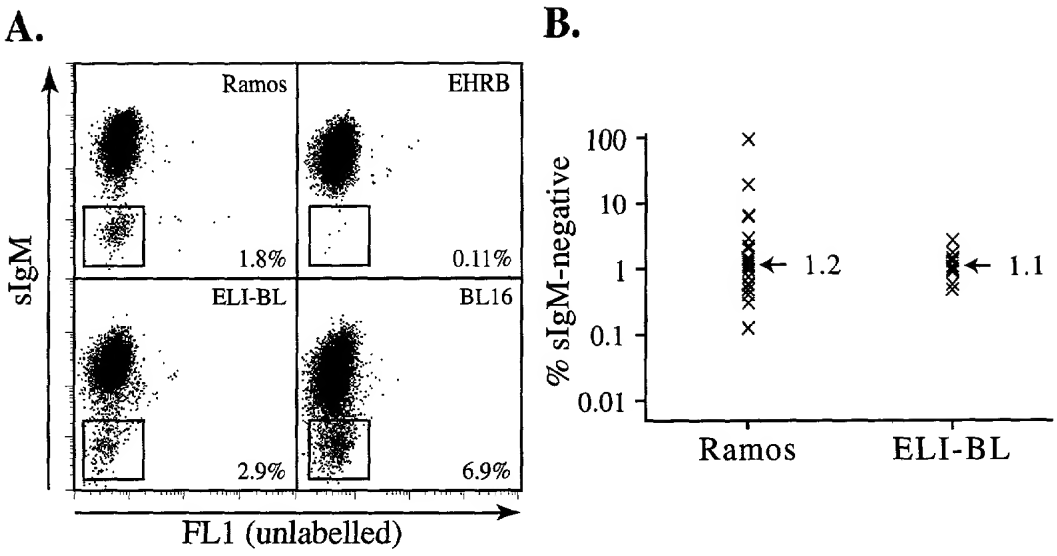
FIGURES 20A-C



FIGURES 21A-D



FIGURES 22A-B



C.

```
GTG CAG CTG GTG GAG TCT GGG GGA GGC GTG GTC CAG CCT GGG GGG TCC CTG AGA CTC TCA TGT GCA
V   Q   L   V   E   S   G   G   G   V   V   Q   P   G   G   S   L   R   L   S   C   A
1                                   10                              20

                    t   a g   g
GCC TCT GGA TTC ACC GTC AGT AGC AAC TAC ATG ACC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG
A   S   G   F   T   V   S   S   N   Y   M   T   W   V   R   Q   A   P   G   K   G   L
                            30                              40

      t                     a   c   ac  t   c  cg  t                t   cc
GAG TGG GTG TCA CTT ATT TAT AGC GGT GGT AGC ACA ACA TAT TAC GCA GAG TCC GTG AAG GGC CGA
E   W   V   S   L   I   Y   S   G   G   S   T   T   Y   Y   A   E   S   V   K   G   R
                50                              60

      t   c                         t a                     t   g   a
TTC ACC ATC TCC AGA GAC AAT TCC AAA AAC ACG ATG TAT CTT CAA ATG AAC AGC CTG AGA GTA GAG
F   T   I   S   R   D   N   S   K   N   T   L   Q   M   N   S   L   R   V   E   D   T
            70                              80

      t       a                         a   t   a                       g
GAC ACG GCT GTG TAT TAC TGT GCG GGA GAC CTG AAC AGC ACC TCG GTA GGG ACT AAT AAT TTC TAC
M   N   S   V   R   V   E   D   T   A   V   N   S   T   S   V   G   T   N   N   F   Y
        90                              100                             110

ATG GAC GTC TGG GGC AAA GGG ACC ACG GTC ACC GTC TCC TCA
M   D   V   W   G   K   G   T   T   V   T   V   S   S
                                    120
```

FIGURES 23A-C